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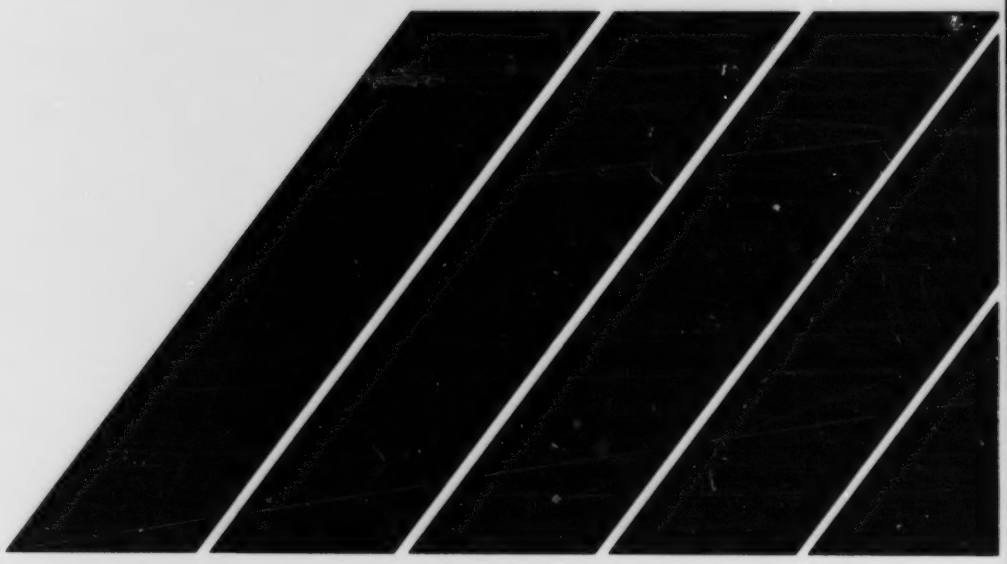
QUALITATIVE AND QUANTITATIVE ASSESSMENT OF FUNGI ASSOCIATED WITH WOOD CHIP FUEL PILES



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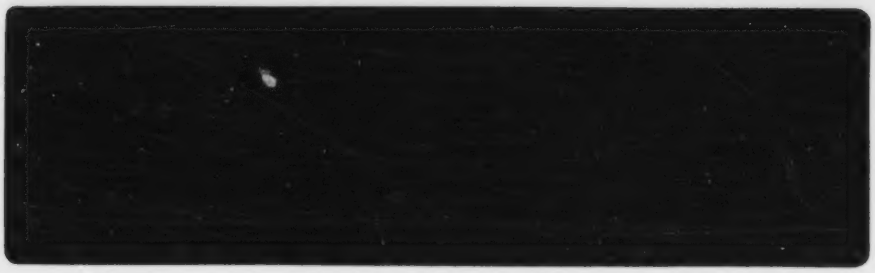

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QUALITATIVE AND QUANTITATIVE ASSESSMENT OF FUNGI ASSOCIATED WITH WOOD CHIP FUEL PILES

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Executive Summary

The emerging use of wood chips as an alternate energy source has led to an increase in the number of wood chip piles in domestic and institutional environments. The presence of these piles may have an adverse effect on the quality of the air around them because of microorganisms surviving or growing on the wood.

Depending on the type of wood used and the harvesting, handling and storage conditions fuel wood chips have been found to support growth of a wide range of microorganisms, particularly fungi.

In indoor storage areas of wood chips spores, cells and cell particles can become airborne and be inhaled by persons working in these areas. A number of methods exist to sample and monitor airborne microbial particles. These methods, particularly if two or more are combined, are suitable to detect and quantify viable and non-viable airborne microbial particles, to determine particle sizes and, in most cases, to identify the microorganisms collected to the species level.

Many of the fungi, actonomycetes and other bacteria which have been reported to occur on wood chips are pathogenic or toxigenic. Inhalation of microbial spores, cells or fragments, especially at high concentrations and small particle sizes, can lead to serious allergic reactions or pulmonary diseases. There is evidence that even low concentrations of microbial aerosols may have sub-acute long-term health effects.

Some microorganisms appear to produce toxins in sufficient concentration that toxicoses can be caused by them via the inhalation route. Microbially produced volatiles have also been suspected to cause adverse health effects.

Proper harvesting, storage and handling procedures can minimize the contamination of wood chips by microorganisms and subsequent contamination of the air. However, it appears that wood chips are almost never free of potentially harmful microbial species for which safe levels still need to be established. It is therefore recommended that persons working in wood chip storage areas or handling wood chips indoors should wear suitable protective clothing and respirators.

Résumé

La nouvelle technique d'utilisation des copeaux de bois, comme autre source d'énergie, a entraîné une augmentation de nombre d'amas de copeaux dans des propriétés privées et publiques. Cela peut contribuer à la détérioration de la qualité de l'air environnant en raison des micro-organismes qui subsistent dans ces amas ou s'y développent.

On a découvert que, selon le type de bois et les méthodes de coupe, de manutention et de stockage utilisés, les copeaux de bois servant de combustible favorisent la croissance d'une foule de micro-organismes, surtout de champignons.

Dans les entrepôts de copeaux de bois, il peut y avoir des spores, des cellules et des particules cellulaires en suspension dans l'air et les travailleurs affectés dans ces endroits risquent d'inhaler ces organismes. Il existe quelques méthodes d'échantillonnage et de surveillance des concentrations de particules microbiennes en suspension dans l'air. Ces méthodes, surtout si on en combine deux ou plusieurs, s'avèrent efficaces pour déceler la présence de particules microbiennes viables et non viables en suspension dans l'air, pour en déterminer les concentrations et les dimensions et, la plupart du temps, pour identifier les espèces de micro-organismes recueillis.

Bon nombre de champignons, d'actinomycètes et d'autres bactéries dont on a relevé la présence dans les copeaux de bois sont pathogènes ou toxigènes. L'inhalation de spores, de cellules ou de fragments microbiens risque de provoquer de graves réactions allergiques ou des maladies pulmonaires, surtout si ces particules se présentent en fortes concentrations et si elles sont de petites dimensions. Le recherche a montré qu'à long terme, même des concentrations réduites d'aérosols microbiens peuvent avoir des effets subaigus sur la santé.

Quelques micro-organismes semblent produire des toxines en concentrations assez fortes pour que leur inhalation provoque des toxicoses. Il semblerait même que des volatiles produits par voie microbienne peuvent avoir des effets néfastes sur la santé.

L'utilisation de méthodes appropriées de coupe, de stockage et de manutention peut contribuer à la réduction des risques de contamination de copeaux de bois par les micro-organismes et, donc, de l'air. Cependent, il semble que les copeaux contiennent presque toujours des espèces microbiennes nocives pour lesquelles il faudra déterminer la marge de sécurité. On recommande donc le port de vêtements et de masques de protection aux personnes qui travaillent dans des entrepôts de copeaux ou qui font la manipulation de ce produit utilisé comme source d'énergie.

Introduction

In recent years, there has been an increasing interest in the use of wood chips not only for residential heating but also as fuel for certain industries such as commercial greenhouses and institutions such as schools and hospitals. This development was initiated by the increase in oil prices in the 1970's when the cost of wood became competitive with the cost of fossil fuels. More recently, concerns about the increase in atmospheric carbon dioxide and the expected resulting "greenhouse effect" has given the use of fuel from renewable resources another impetus. Increased use of renewable energy sources is encouraged in the hope that this will slow the build-up of "greenhouse gases".

Although the use of fuel wood chips presents new opportunities for the forest industry, it also creates problems as an unfamiliar product is introduced into the indoor environment. Depending on the condition under which they are stored wood chips are subject to colonization by a wide range of microorganisms. Especially fungi growing in wood chip piles have become a cause of concern since several cases of adverse health effects have been related to exposure to fungal aerosols in indoor environments where wood chips were stored and handled. Exposure to airborne microbial spores, cell fragments, volatile products, etc. is known to potentially result in allergic reactions, toxicoses or infections. However, there is, as yet, little to guide the analyst in the best ways to sample the air in a building for microorganisms and how to interpret the results obtained. There is also little information available on the methods of handling and storage which are best suited to reduce the risk of human exposure to microorganisms originating from wood chips.

The objective of this report is to gather existing information on determining microorganisms in indoor air in relation to potential hazards posed by wood chip storage and to present recommendations with regard to the safe handling and storage of wood chips in indoor environments.

Fungi and Other Microorganisms Growing on Wood Chips

Under certain conditions, especially in the presence of moisture, wood is a good substrate for microbial growth. Many fungi are particularly well adapted to colonize wood. If trees are converted into chips of various sizes, the surface area is greatly increased inviting even more extensive microbial growth (Lindgren and Eslyn 1961, Eklund et al. 1973). While in the pulp industry wood chips are normally made from debarked round wood for heating purposes, often whole trees or logging residues are being used which include bark and needles. This results in a mixture relatively rich in nutrients which is even more favourable for microbial growth (Thörnquist and Lundström, 1982; Zoch et al. 1982).

There has been an interest in microbial growth in wood chip piles for many years. This interest arose mainly from concern about the deterioration of chips for use in the pulp and paper industry (Smith 1973, Ofosu-Asiedu and Smith 1973, Eklund et al. 1973). Large wood chip piles often heat spontaneously due to microbial activity and thermotolerant and thermophilic fungi are often responsible for fibre loss and discoloration of the chips (Springer and Hajny 1970, Tansey 1971, Shields and Shih 1971). The spontaneous heating process often leads to charring or even ignition of the chips (Tansey, 1971, Zoch et al. 1982).

Numerous studies have been concerned with the composition of microbial populations in wood chip piles, the most extensive being the ones by Shields (1969) and Hoover-Litty and Hanlin (1985). In the two studies, a total of 142 different genera of fungi and 221 species are reported from various types of wood chips. While the total number of species colonizing wood

chip piles can be enormous, the fungal population is usually dominated by relatively few genera and species. Hoover-Litty and Hanlin (1985) list the seven most commonly found genera as Trichoderma, Fusarium, Chaetomium, Aspergillus, Rhizopus, Graphium, and Penicillium. By far the most dominant genus was Trichoderma which formed almost 20% of all colonies recovered.

Many other workers who studied fungal populations in wood chip piles similarly report relatively few prevalent fungal genera and species. Some of these are the same as the ones reported by Hoover-Litty and Hanlin, e.g. Aspergillus, Penicillium, Trichoderma and Chaetomium. However, not unexpectedly, the fungal composition varies with the type and history of the wood chips studied and with the methods of sampling and isolation used. For instance Thörnquist (1983) reported that the fungal population changes over the storage time with the location within the pile and with the availability of nutrients. During periods of self heating of the pile, thermotolerant and thermophilic species are being found in greater numbers while mesophilic species dominate when the storage pile is near ambient temperature. All these variables account for the differences in the composition of fungal populations in wood chip piles reported by various authors. However, despite the variability of the microbiology of wood chips, potentially hazardous fungi are being found regularly. For example, of the twenty-three publications reviewed in this report which deal with fungi growing on wood chips, twenty-one (91%) report the presence of the genus Aspergillus and eighteen (78%) the presence of A. fumigatus (Table 4).

Moist wood chips are readily colonized by fungi and most heavy fungal contaminations occur during periods when the water content of the chips is high. However, many fungi are able to germinate, grow and sporulate at relatively low water activities (Ayerst 1969, Magan and Lacey 1984). It can therefore be expected that even under relatively dry conditions some fungi will grow and produce spores. Magan and Lacey (1984) found that some Aspergillus and Penicillium species were xerophilic. Many of the studies related to xerophilic fungi have been concerned with fungi growing on dried and stored food items (Hocking and Pitt 1980, Dallyn and Fox 1980) where economic and health aspects are particularly important. However, many wood inhabitating fungi can be expected to survive under relatively dry conditions as the study by Zimmerman and Butin (1973) indicates. Fungi are not as dependent on the moisture content of the wood as on the relative humidity of the air within the chip pile. As a result some fungi grow on wood with a moisture content of 10% when the relative humidity of the air is high (Thörnquist and Lundström 1982). For the detection of xerophilic fungi the use of nutrient media with low water activity may be necessary (Samson 1985).

Although fungi appear to be the most common microorganisms found, bacteria and actinomycetes are also able to grow in large numbers on wood chips under certain conditions. Bacteria require a higher moisture content in the chips than fungi. Feist et al. (1973) showed bacterial populations in wood chip piles as high as 5×10^8/gram of dry wood. They found that bacteria contributed significantly to the self heating of the pile. Although not very often reported, actinomycetes are likely to be present frequently especially if the wood chip pile has gone through a period of self heating. Pellikka and Kotimaa (1983) found Streptomyces sp. and Thermoactinomyces vulgaris in piles of birch and alder chips which had a history of self

heating. Lundgren and Rosenhall (1979) and Helvoe and Ostberg (1980) present serological evidence that patients with allergic alveolitis after handling wood chips had been exposed to the actinomycete Micropolyspora faeni.

Sampling of Microorganisms

In order to assess the potential health hazard due to microorganisms growing on wood chips, it is important to monitor microbial populations on the chips as well as microbial particles released from the chip pile into the air.

Microorganisms growing on chips are typically assessed qualitatively and semi-quantitatively by placing wood particles directly on or in nutrient media or by washing microbial propagules off the chips and plating a quantity of the suspension on suitable nutrient media (Miller et al. 1982). Counting and identification of the resulting colonies will give some information on the degree of contamination and the presence of potentially harmful organisms.

For the sampling of airborne viable and non-viable microbial particles a wide range of methods and sampling equipment is available. To assess the health hazard of airborne microbial particles a knowledge of particle size distribution, density and species composition is important and the proper collection procedure has to be selected depending on the conditions and the type of microorganisms expected. Often it may be necessary to use more than one procedure. A number of authors have reviewed the various types of samplers available (e.g. Burge et al. 1987, Chatigny 1983, Muilenberg 1989).

A simple way of sampling airborne microbial particles is by gravity collection on nutrient agar plates or on surfaces coated with adhesive such as microscope slides which are exposed to the environment to be tested. This method is easy to perform. However, it has its limitations as it tends to be heavily biased towards denser particles.

There are three types of more sophisticated samplers. The largest group are the ones which operate on the impaction principle.

Rotating arm impactors function by moving collection surfaces through the air. These can be rods or slides coated with a suitable adhesive. The speed of the rotating collection surfaces can be controlled thereby allowing the calculation of the volume of air to which they are exposed.

Suction impactors use a pump or vacuum source to accelerate air through an orifice or sieve thereby increasing particle momentum. Particles are then impacted on an agar plate or adhesive surface depending on whether a count of colony forming units of viable particles (cfu) or a total particle count is to be performed. Some of the impactors are of the multi-stage or cascade type which allow the collection of fractions of different particle size separately. A recently developed portable sampler uses centrifugal forces for the impaction of microbial aerosols on nutrient medium (Macher and First 1983).

While the sampling capacity of impactors is limited by the surface of their collecting area, samplers working on the principle of the liquid impinger are less restricted in this respect. In these samplers the incoming air stream is directed into a liquid in which the particles are collected. The collection of hydrophobic particles, such as fungal spores, presents a problem with this sampling method which can be overcome by adding surfactant to the collection liquid. Liquid impingers normally consist of various stages for particle size fractionation. Particles in the liquid can either be inspected and counted microscopically or plated on nutrient medium

for colony counts.

Filter cassettes have also proven useful for the collection of microbial aerosols (Palmgren et al. 1986a, 1986b). Air is drawn through vacuum filters of known pore size and particles retained on them can either be inspected directly or washed off for further processing. If gelatine filters are used these can be dissolved after sampling, and the particles released into the liquid for subsequent inspection or plating.

Most impactors, liquid impingers and filter samplers are suitable for volumetric sampling, yielding results as particles/m^3 or cfu/m^3. However, in the interpretation of these data it has to be kept in mind that samplers vary in their efficiency. While some of the samplers require stationary pumps or vacuum sources for operation others are battery operated and portable. Selection of a sampler or sampling technique depends on a number of factors such as the reason for the survey, the types of microorganisms present , density of the microbial aerosol, location etc. Table 1 summarizes the most common types of samplers together with their characteristics. Appendix 1 gives more detailed information and illustrations of these devices.

A number of authors have compared samplers with regard to their suitability for various applications in monitoring microbial aerosols.

A comparison of the standard six-stage Anderson sampler and the modified one-stage (N-6) Anderson sampler was performed by Jones et al. (1985). Using rose bengal streptomycin (RBS) growth medium which is selective for fungi, it was demonstrated that the two samplers

TABLE 1

Samplers Frequently Used for Sampling Microbial Aerosols

Sampler	Principle of Operation	Sampling Rate (l/min)	Sampling Time	Applications Remarks
Open petri dish with nutrient agar	Gravity settling	-	0 to 4 hrs	Biased to collect large particles (CFU)
Open settling surface coated to hold particles	Gravity settling	-	Unlimited	Same as above
Rotorod	Rotating arm impactor	47.3 paired rods	1 min to ≈ 48 hrs	Good for particles >10μm
Burkard recording outdoor	Suction slit impactor	10	Up to 7 d	Time discrimination down to about 2 h
Burkard personal	Suction slit impactor	10	Up to 30 min	DC battery holds charge for <45 min
Slit to agar impactor	Impaction on agar on rotating plate	30-700	Depends on model and circumstances	Provides information on aerosol concentration over time. Bulky, AC operation
Andersen six-stage sieve-type	Impaction on nutrient agar	28	1 min Min. 20 min Max.	Collects CFU unless surfaces are washed into medium. Provides particle-size data.
Andersen 2-stage sieve-type	Impaction on nutrient agar	14-28	1 min Min.	Divides sample into respirable and non-respirable fractions
Andersen single-stage (N-6) sieve-type	Impaction on nutrient agar	28	1 min	AC operation, useful as probe
SAS sampler	Impaction on "rodak" plate	90 or 185	1/2 or 1/3 min	Portable; high flow rate; less efficient and uses smaller plate than Andersen
RCS sampler	Impaction on agar in plastic strips	40	1/2 min	Small, portable, useful as a probe
All Glass Impingers	Impingement into liquid	Depend on model	varies	Fungi require wetting agent; useful over wide range of particle concentrations
Filter Cassettes (5,7)	Filtration	2 - ?	Min to h	Some desiccation loss; portable, inexpensive, useful in high concentration areas

were comparable up to a concentration of fungal particles of 3000 cfu/m^3. Disadvantages of the N-6 sampler are that it does not provide size distribution information and it is prone to overloading in areas of high concentrations of fungal aerosols.

The Anderson six-stage sampler was compared with the two stage version with regard to their efficiency in measuring bacterial and fungal viable aerosols (Gillespie et al. 1981). Values obtained with the two-stage sampler were lower than with the six-stage sampler when the concentration of particles greater than one μm was less than 1000/m^3.

The two stage Anderson sampler and the May three stage glass impinger were compared by Zimmerman et al. (1987) with regard to their efficiency in collecting aerosols of Escherichia coli. Results with both samplers appeared to be fairly well correlated. At high aerosol concentrations (>300 cfu/m^3) the Anderson sampler tended to become overloaded. However the Anderson sampler was much easier to operate compared with the lengthy procedure required for the May sampler.

Lundholm (1982) compares sampling methods for quantitative determination of viable airborne bacteria. Samplers compared were the Casella slit sampler, the Anderson sampler, the all-glass impinger, gelatin filters (Sartorius) and cellulose membrane filters (Millipore). The Anderson sampler consistently gave the highest counts. Desiccation of cells was a problem with the filter methods.

Kang and Frank (1989) compared an all-glass impinger (AG1-30), the RCS sampler, Anderson 6 - stage sampler and millipore filter for their efficiency to sample airborne bacteria and bacterial endospores. They found that the Anderson impactor was the sampler of choice and that the RCS sampler was least efficient in sampling bioaerosols of small-sized particles such as bacteria or endospores. The American Conference of Governmental Industrial Hygienists (Morey, et al. 1986) recommends the Anderson sampler as a reference instrument for sampling airborne viable microorganisms in indoor environments.

Verhoeff et al. (1988) compared the efficiency of four samplers in combination with three nutrient media to enumerate and identify viable fungal particles in houses. At relatively low concentrations (mean cfu/m^3 ranging from 84-3239) the slit-to-agar and the N-6 Anderson samplers were found to perform better than the SAS and RCS sampler. The use of the N-6 Anderson sampler was recommended because it is easier to use than the slit-to-agar sampler.

Smid et al. (1989) compared four samplers in occupational environments with fungal aerosol concentrations of between 10 and 3700 cfu/m^3. The Anderson N-6 sampler and the slit-to-agar sampler were the most efficient. The RCS sampler gave good results also and proved to be more practical to use. The SAS sampler tended to underestimate fungal concentrations.

Blomquist et al. (1984a, 1984b) used modified slit samplers (Casella, BIAP) for counting high concentrations of viable airborne fungal particles (>10^5 cfu/m^3) since they found that the Anderson cascade impactor was unsuitable for counts at these concentrations. Filter methods

using gelatine gel filters were also suitable to sample high concentrations of viable fungal aerosols.

Lembke et al. (1981) determined the precision of the all-glass impinger (AGI-30) and the Anderson microbial impactors for air sampling. Colony counts obtained with impingers indicated a linear relationship between samplers over an observed aerosol concentration of 1.1 x 10^3 - 2.5 x 10^7 cfu/m^3. Impactors indicated a linear relationship over a 3.9 x 10^3 - 1.9 x 10^5 cfu/m^3.

Burge, et al. (1977) compared total spore counts obtained with the Burkhard automatic volumetric sampler with viable fungal spore counts conducted with the Anderson sampler. At low spore levels (<100/m^3) viable counts were 20-40% of the total counts. At higher levels (>500/m^3) only about 5% of the spores were counted with the Anderson samples.

Delmore and Thompson (1981) report a higher sampling efficiency at low microbial aerosol concentration of the RCS sampler compared to a slit - to agar sampler and an all-glass impinger.

In a detailed examination of the RCS sampler Macher and First (1983) showed that efficiency of the RCS sampler decreased sharply with decreasing particle size (Fig. 1). However they concluded that its ease of operation makes it a useful instrument for field survveys information, although it is not the sampler of choice for quantitative estimates of microbial aerosols.

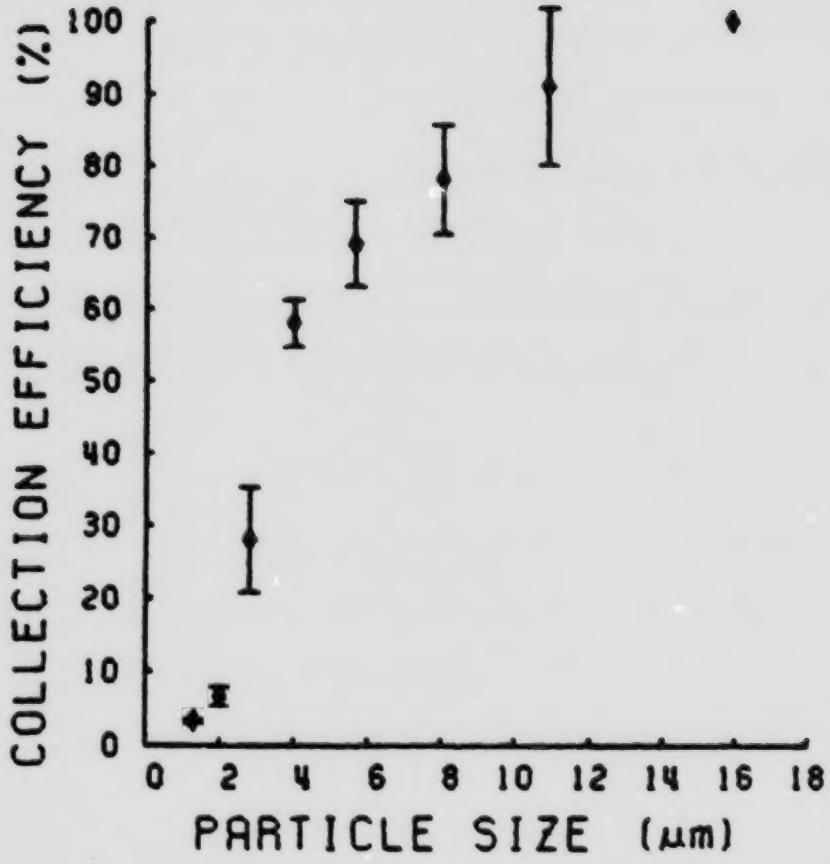

Fig. 1 Collection efficiency of the RCS sampler. Vertical bars represent standard deviations (Macher and First 1983)

Lach (1985) compared the SAS sampler and the Bourdillon slit samples at low levels of airborne microorganisms. They found that the SAS sampler is less efficient at particle sizes $<4\mu m$.

Under conditions of high levels of fungal and bacterial aerosols (10^3 - 10^5 cfu/m^3) at a waste disposal site Crook et al. (1988) found that the Millipore filter method gave the best results, followed by the multi-stage liquid impinger. The Anderson 6 stage sampler was least efficient.

Palmgren et al. (1986a, 1986b) found that the Nuclepore filter method was suitable for quantitative analysis of highly concentrated bacterial and fungal aerosols. They used Nuclepore filters (0.4μm pore size, 37 mm diameter, Millipore filter cassettes, Millipore Corp. Bedford, MA) to enumerate and classify airborne fungi, bacteria and actinomycetes at aerosol concentrations up to 10^{11} particles /m^3.

Health Implications Related to Microorganisms Growing on Fuel Wood Chips

1.) Direct Effects of Microorganisms

Since fungi are the microorganisms most frequently reported to be associated with wood-chips and other lignocellulosic materials this section of the report will be primarily concerned with fungi and their potential health effects.

However, other microorganisms such as bacteria including actinomycetes have also been isolated from wood chips (Pellikka and Kotimaa 1983). Airborne spores of actinomycetes are known to cause severe illnesses such as "farmer's lung", a hypersensitivity pneumonitis typically caused by exposure to mouldy hay (Emanuel and Kryda 1983). Under certain conditions actinomycetes can grow in wood chip piles and must therefore be considered a potential hazard. There is little detailed knowledge on other bacterial species colonizing wood chips. However it has been suggested that endotoxin containing gram negative bacteria could be released from wood chips under some conditions and pose a health hazard when inhaled.

Fungi are known to affect human health in various ways. Three basic types of diseases caused by fungi can be distinguished:

Mycoses are the result of the invasion of living tissue by fungi. Fungi which are able to invade- or infect- human tissue often are thermophilic or thermotolerant with optimal growth rates at or above body temperature. Aspergillosis (Pepys 1969) and mucormycosis (Tintelnot

and Nitsole 1988) are examples of fungal infections. However, relatively few fungi invade tissue of healthy people. Fungal infections almost always require some compromise of the immune system (Burge 1987).

Toxicoses are caused by toxic secondary metabolites of fungi. Such metabolites are produced by many fungi and can have severe chronic and acute health effects. If such compounds are taken up either by ingestion or inhalation they can affect various organ tissues e.g. kidneys, liver, lungs etc. Well known examples of mycotoxins are trichothecenes and aflatoxins.

Sensitization of tissue to fungal proteins or polysaccharides can lead to allergies. Fungal spores or mycelial material which comes in contact with human tissue often causes this type of hypersensitivity response (Burge 1986, Salvaggio and Aucraft 1981). Herxheimer et al. (1969) and Lehrer et al. (1986) present evidence that fungal spores in the outside air are responsible for many cases of respiratory allergies. Fungi which have been reported as allergenic are listed in Table 2.

Diseases such as the ones mentioned above can be caused by fungi and other microorganisms growing on wood chips. The route of uptake of microbial spores or vegetative cells in indoor environments where wood chips are stored or handled is usually by inhalation. Spore size of the allergenic organisms has an impact on the effect and the type of disease caused by inhalation. As shown in Fig. 2, the severity of the health effect increases with decreasing spore size. Spores of a diameter of about 10μm or larger normally don't penetrate

TABLE 2

Fungi and Actinomycetes Reported as Allergenic[1.)]

Myxomycetes
- *Fuligo*
- *Stemonitis*

Phycomycetes
- *Mucor*[2.)]
- *Absidia*
- *Rhizopus*[2.)]

Ascomycetes
- *Xylaria*[2.)]
- *Eurotium*
- *Erysiphe*
- *Daldinia*
- *Chaetomium*[2.)]
- *Didymella*
- *Claviceps*
- *Microsphaera*
- *Saccharomyces*[2.)]

Basidiomycetes
- *Tilletiopsis*
- *Puccinia*
- *Tilletia*
- *Rhodoturola*
- *Sporobolomyces*
- *Ustilago*
- *Urocytis*
- *Dacrymyces*
- *Agrocybe*
- *Amanita*
- *Boletus*
- *Nematoloma*
- *Psilocybe*
- *Chlorophyllum*
- *Podaxis*
- *Agaricus*
- *Armillaria*
- *Coprinus*[2.)]
- *Hypholoma*
- *Ganoderma*
- *Merulius*[2.)]
- *Polyporus*[2.)]
- *Stereum*[2.)]
- *Pleurotus*
- *Cantharellus*

Deuteromycetes
- *Alternaria*[2.)]
- *Cladosporium*[2.)]
- *Aspergillus*[2.)]
- *Sporobolomyces*[2.)]
- *Penicillium*[2.)]
- *Epicoccum*[2.)]
- *Fusarium*[2.)]
- *Candida*
- *Rhodotorula*
- *Tilletiopsis*
- *Phoma*
- *Botrytis*
- *Helminthosporium*
- *Monilia*
- *Paecilomyces*[2.)]
- *Sporotrichum*[2.)]
- *Stemphylium*
- *Trichothecium*
- *Cephalosporium*
- *Gliocladium*[2.)]
- *Aureobasidium*[2.)]
- *Trichoderma*[2.)]
- *Coniosporium*
- *Dicoccum*
- *Nigrospora*
- *Acremonium*
- *Drechslera*
- *Torula*[2.)]
- *Spondylocladium*
- *Curvularia*[2.)]
- *Epidermophyton*
- *Trichophyton*

Actinomycetes
- *Micropolyspora*
- *Thermoactinomyces*

1.) From: Burge (1985, 1989), Lacey et al. (1972).
2.) Found on wood chip piles.

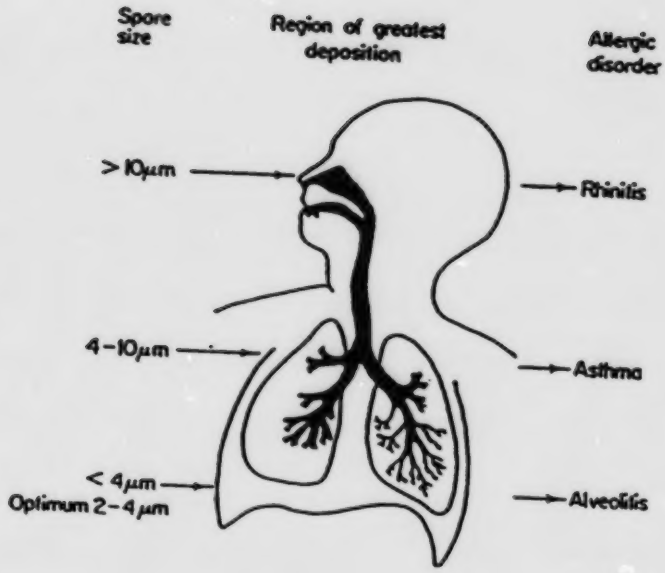

Fig. 2 The effect of spore size on lung penetration and the resulting type of disease (Lacey et al. 1972)

very deep into the respiratory system and their effect is restricted to milder forms of irritation, such as rhinitis, as they are deposited in the nasal and pharyngal region. Particles in the size range of (10 -4μm) tend to penetrate deeper into the trachea, bronchi and bronchiole. Spores or other microbial particles deposited in this region may cause asthmatic responses. Finally, particles smaller than 4μm are able to penetrate into the alveoli causing alveolitis under certain conditions. Figure 3 shows relative spore sizes of some allergenic and pathogenic fungi.

A number of cases of pulmonary diseases related to wood-borne microorganisms have been reported (see Table 3).

Hedenstierna et al. (1986) found restrictive impairment of lung function in wood trimmers which appeared to be related to exposure to moulds originating from wood. Dust and air in the work area was found to be heavily contaminated with mould and fungal spores particularly of the genus Rhizopus. Antibodies to Rhizopus were found in the sera of many workers.

Wiemander and Belin (1980) noted the similarity between the symptoms of "farmers lung" disease and symptoms experienced by wood trimmers who worked in mould contaminated environments. In a comparison of sawmills with and without mould problems Belin (1980) found a much higher percentage (55 vs. 7%) of immune responses to mould extracts in workers working in the contaminated mills. Fever reactions occurred in workers from the contaminated environments only.

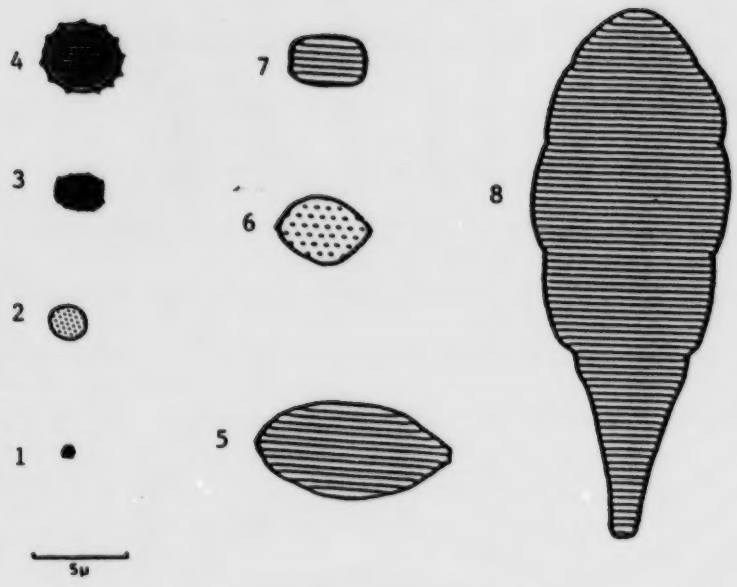

Fig. 3 Spore sizes of some allergenic and pathogenic fungi (After Pepys 1969)

1. *Thermoactinomyces vulgaris* Tsiklinski
2. *Mucor pusillus* Lindt
3. *Aspergillus fumigatus* Fresenius
4. *Aspergillus niger* Van Tieghem
5. *Cladosporium herbarium* Fries
6. *Chaetomium funicola* Cooke (Ascospore)
7. *Cryptostroma corticale* (Ellis and Everhart) Gregory and Waller
8. *Alternaria sp.*

TABLE 3

Occupational Hypersensitivity Diseases of the Lungs.

Disease	Source of Antigen	Causative Agents (Confirmed or suspected)	Reference
Farmer's Lung	Mouldy Hay	Micropolyspora faeni Thermoactinomyces vulgaris	Pepys 1969
Mushroom Workers' Lung	Mushroom compost	M. faeni, T. vulgaris	Sakula 1967
Maple Bark Disease	Mouldy maple bark	Cryptostroma corticale	Emanuel et al. 1962
Sequoiosis	Mouldy sawdust	Graphium Aureobasidium pullulans	Cohen et al. 1967
Pulpmill Worker's Disease	Mouldy pulpwood	Alternaria sp.	Schlueter et al. 1972
Sawmill Worker's Lung	Mouldy wood	T. vulgaris, Aspergillus sp.	Terho et al. 1980
Wood Trimmer's Disease	Mouldy timber	Rhizopus sp. Aspergillus fumigatus	Wimander and Belin 1980
Woodchip Handler's Disease	Mouldy wood chips	Aspergillus sp., Mucor sp., M. faeni	Lundgren and Rosenhall 1979

In a more extensive survey of wood trimming departments of sawmills in Sweden, Belin (1987) found about 100 workers who had shown clinical symptoms such as fever, muscular pain, malaise and cough. Forty of these workers had their disease formally accepted as occupational disorder. Many of the wood-trimming departments were found to have mean values of air contamination by moulds exceeding 10^6 cfu/m^3. At these levels more than 50% of the workers were found to form precipitating antibodies and between 10 and 20% showed febrile reactions. Fungi found growing on the wood were primarily Rhizopus rhizopodiforme, Paecilomyces variotii and Aspergillus fumigatus.

Terho et al. (1980) reports a case of extrinsic allergic alveolitis in a sawmill worker. From environmental and immunological tests it appeared that either Aspergillus fumigatus or Thermoactinomyces vulgaris was the causative agent.

A rare case of pneumonitis due to inhaled spores of the fungus Cryptostroma corticale was first reported by Towey et al. (1932). This fungus was found to grow and sporulate underneath maple bark and workers were exposed to the airborne spores during the debarking process. The causative agent was confirmed later in similar cases reported by Emanuel et al. (1962) and by Wenzel and Emanuel (1967).

Two cases of hypersensitivity pneumonitis in workers employed in a pulp mill were described by Schlueter et al. (1972). Fungi of the genera Penicillium, Alternaria, Aspergillus, Trichoderma and Rhizopus were isolated from dust in the work environment. However, inhalation challenge and serological tests indicated that Alternaria was at least a major

causative agent in these patients' illness.

Lundgren and Rosenhall (1979) report a case of allergic alveolitis due to handling of mouldy fuel wood chips. The serum of the patient contained precipitating antibodies against Aspergillus, Mucor and M. faeni.

Two cases of illness closely resembling the so called "wood trimmer's disease" were reported by Holvoe and Ostberg (1980). These illnesses were apparently related to the use of wood chips in home heating. Serological tests pointed to exposure to several moulds including Rhizopus, Mucor, Paecilomyces, Botrytis, Penicillium and the actinomycete M. faeni.

Five cases of illness caused by exposure to mouldy wood chips are described by Kolmodin-Hedman et al. (1987). Diseases included acute alveolitis, urticaria, chronic bronchitis and atypical respiratory symptoms. A number of mesophilic and thermotolerant fungal species were identified in the work environment including Aspergillus fumigatus, Aspergillus niger, Aspergillus spp., Penicillium spp., Oidiodendron sp., Rhizopus sp., Paecilomyces sp. and Trichoderma sp.

Jappinen et al. (1987) found precipitating antibodies against Aspergillus fumigatus in three out of six workers who handled pine and birch chips outside. Although significant respiratory symptoms were not noted, the workers had a tendency to decreased pulmonary diffusion capacity and vital capacity. Seventeen different species of fungi and yeasts were isolated from the chips. Prevalent were Aspergillus fumigatus, other Aspergilli and Penicillium.

The above mentioned studies show clearly that microorganisms, particularly fungi, growing on wood, bark and wood chips can pose a health hazard particularly when spores, vegetative cells or cell particles become airborne.

Health problems had rarely been apparent in earlier years when wood chips were used in the pulp and paper industry only and were stored and handled outside. However, as the study by Jappinen et al. (1987) shows subacute and immunological effects can be demonstrated even under outside conditions and the possibility of serious health effects as a result of long-term low level exposure cannot be ruled out.

2.) Microbial Toxins

Most evidence for health effects from microorganisms originating from wood chips appears to point to direct effects of cells or cell particles on human tissue. However, there is also evidence that secondary metabolites such as mycotoxins can occasionally be present in airborne microbial particles and be absorbed through the lungs. As pointed out in a report by Tobin et al. (1987) mycotoxins are sometimes found concentrated in fungal spores. Sorensen et al. (1987), for example, provide experimental evidence that fungal aerosols of Stachybotrys atra, a cellulolytic saprophyte, contain dangerous levels of trichothecene mycotoxins. Jarvis and Yatawara (1985) report a case of airborne trichothecene toxicosis originating from S. atra infestation in a house. However, as most known cases of mycotoxins have been due to ingestion of the toxin, toxicological information on the inhalation route of mycotoxins is very limited. Table 4 lists some mycotoxin-producing fungi, many of which have

TABLE 4

SOME GENERA AND SPECIES OF POTENTIALLY TOXIGENIC FUNGI WHICH HAVE BEEN REPORTED TO OCCUR ON WOOD CHIPS

	A. fumigatus	Other *Aspergilli*	*Chaetomium*	*Fusarium*	*Paecilomyces*	*Penicillium*	*Rhizopus*	*Trichoderma*
Eklund et al. (1973)		+				+		
Flannigan and Sagoo (1977)	+						+	+
Frederikson (1985)	+					+	+	+
Greaves (1975)		+		+	+	+		+
Hedenstierna (1986)		+				+		
Hoover-Litty and Hanlin (1985)	+	+	+	+		+	+	+
Hulme (1975)	+		+			+		
Hulme and Stranks (1976)	+		+					+
Jäppinen et al. (1987)	+					+		
Jirjis (1989)	+	+			+	+		+
Kalmodin-Hedman et al. (1987)		+			+	+	+	+
Miller et al. (1982)	+	+				+		+
Nordstrom (1973)	+							
Shields (1966)					+	+		+
Shields (1969)								
Shields and Shih (1971)	+			+	+	+		+
Smith (1973)	+							
Smith and Ofosu-Asiedu (1971)	+							
Tansey (1971)	+		+					
Thörnquist and Lundström (1982)	+				+	+		
Thörnquist and Gustafson (1983)	+	+				+		
Thörnquist (1983)	+	+			+	+		+
Zielinski (1981)	+		+		+	+		+

also been reported to occur on wood chips and other wood substrates.

Flannigan (1987) reviews the occurrence of mycotoxins in the air and evidence from the literature that airborne mycotoxins in domestic and industrial environments may constitute a health hazard. Case reports on the exposure of workers handling grain, corn, peanuts, silage, hay and straw indicate that mycotoxin containing dust can result in pulmonary mycotoxicoses, increased cancer risk, immunosuppression and other chronic health problems. He concludes that there is sufficient evidence in the literature to warrant more research into the toxic potential of airborne dust and moulds in buildings.

There is also evidence that wood-borne fungi are potential sources of mycotoxins, as a study by Land et al. (1987) shows. They found the tremorgenic mycotoxins verruculogen and fumitremorgen in Aspergillus fumigatus present on kiln dried lumber. This led them to conclude that large numbers of spores present in the air in indoor working environments such as sawmills could result in exposure to mycotoxins by inhalation. Since the symptoms during the acutely toxic phase of "wood trimmers' disease" are very similar to the symptoms produced by these tremorgens they concluded that "wood trimmers' disease" and similar occupational diseases are, at least in part, mycotoxicoses.

Endotoxins are pyrogenic lipopolysaccharide cell wall constituents of gram-negative bacteria. These substances are very stable and retain their toxicity even in dead bacteria and their fragments. Under certain circumstances endotoxins can be present in airborne dust particles and, if inhaled in larger quantities, can cause tissue damage. In several work

environments, airborne endotoxins have been linked with health problems. These include agricultural silos, animal housing and animal processing plants, textile mills, wastewater treatment plants and composting operations (Rylander and Vesterlund 1982, Olenchock et al. 1987, Epstein and Epstein 1989). Levels of airborne endotoxin in these environments were found to range from 0.1-14.0 $\mu g/m^3$. Endotoxin concentrations in aerosols are usually determined using the very sensitive Limulus Amoebocyte Lysate assay (Rylander and Vesterlund 1982, Donham 1986).

So far there has been little evidence presented that endotoxin originating from bacteria growing on wood chip piles could pose a health hazard. However, heavy growth of bacteria can occur on wood chips under certain conditions (Fiest et al. 1973) and whole cells and cell particles could become airborne after drying of the chips. Malmberg et al. (1985) studied a number of farmers who had been exposed to mouldy hay, grain and wood chips. They concluded that some of the febrile reactions observed appeared not to be related to mould exposure and might have been caused by endotoxins from gram-negative bacteria.

3.) Odours Produced By Fungi

Fungi often produce characteristic odours which cannot only be unpleasant but also have adverse health effects. Samson (1985) reports that odours produced by moulds have been indicated as the cause of headache, eye, nose and throat irritation and fatigue. He also lists a number of fungal genera which have been reported to produce odour compounds. These include Penicillium, Aspergillus, Trichoderma, Alternaria and Fusarium. Although fungi are known to produce a wide range of volatile compounds, relatively little information is

available on the effect of inhaled fungal volatiles on humans (Miller et al. 1988, Tobin et al. 1987).

Handling and Storage of Fuel Wood Chips

Miller et al. (1982) stressed the importance of the history of wood chips prior to indoor storage. They showed that chips made from dry round wood and stored under dry conditions were much less contaminated by fungi than wet chips. Wet chips tended to heat spontaneously in storage and would contain higher concentrations of the more dangerous thermotolerant and thermophilic fungi.

In a detailed study on microbial aerosols originating from fuel wood chips, Pellikka and Kotimaa (1983) came to similar conclusions. They found that spore concentrations in the pile increased during 20 weeks indoor storage. Chips harvested in the summer were more susceptible to microbial growth than chips harvested during the rest of the year. Fine chips were found to be more heavily colonized by fungi than block chips. They point out that wood well dried before chipping would be less subject to microbial growth. Once chips are infested, however, they are less likely to release spores into the air if they are kept moist. If contaminated chips are dried in storage, spores become more easily airborne and the microbial aerosol concentration increases significantly particularly during handling.

Thörnquist and Lundström (1982) surveyed 64 buildings heated with wood chips. Fungal propagules in the air were much higher in houses using wood chips as fuel than in houses heated with oil or logs. After disturbance of the wood chips, the number of fungal particles increased further. Chips from sawmill slabs and from limbed trees usually resulted in lower numbers of fungal particles. They found that with extended storage time, the number

of fungal particles increased in the air of buildings in which the chips were stored. In wood chip piles stored less than one month and more than six months mesophilic fungi dominated. Storage periods longer than one and less than six months favoured the growth of thermophilic and thermotolerant fungi. High moisture content and greater content of hardwood and unlimbed trees and tops favoured an increase in temperature and fungal growth. Proper drying of logging residue has been shown to prevent dry matter losses and excessive microbial growth (Thörnquist and Gustafsson 1983).

Jirjis (1989) compared fuel wood chips from logging residue and from oak wood stored inside and outside for a period of six months. Thermophilic and thermotolerant fungi were dominant in the piles containing chips from logging residue and high numbers of spores were counted. In the oak chip piles, mainly mesophilic and thermotolerant fungi developed and the total spore counts were less. In this study indoor storage appeared to be more favourable for fungal growth than outdoor storage.

Bjorklund (1983) compared the behaviour during storage of chips of different sites and from whole trees vs. limbed trees. The largest chips (70 mm) and the chips from limbed trees self heated very little. The smallest chips (8 mm) and whole tree chips heated most. Total fungal particles were similar in all piles. Chip size and tree species appeared to have little influence on fungal growth.

Several authors discuss possibilities of preventing fungal growth in wood chip piles. Frederikson (1985) stresses the importance of using properly dried limbed raw material and

well ventilated storage facilities for the chipped material to prevent fungal growth and loss of dry mass. Eslyn (1973) tested propionic acid as a biocide to prevent microbial deterioration and self heating of wood chips. He showed that 8 kg of propionic acid per ton of wood chips prevented fungal degradation. This was considered not economically attractive for use in the pulp and paper industry. However, its use would be justifiable under some conditions to prevent unwanted growth of microorganisms on fuel wood chips. Another chemical which showed promise in preventing microbial deterioration of wood chips is borax (sodium borate) (Hulme and Shields 1973). Excluding air from stored wood chips is an effective method of preventing the growth of fungi and other aerobic microorganisms. This can be achieved by compacting the wood chip pile or by storing the chips in an airtight silo (Shields 1975). Air can also be excluded by encasing wood chip piles in polyethylene membranes (Feist et al. 1971).

Conclusions and Recommendations

1.) Sampling and analysis of biological aerosols originating from wood chips stored indoors.

There is still a lack of guidelines and standard procedures for the sampling and evaluation of biological aerosols in indoor environments. Government agencies and researchers have become increasingly interested in this topic and information is being accumulated which will eventually lead to the establishment of guidelines (Miller et al. 1988, Tobin et al. 1987, Morey et al. 1986). However, most of the studies done so far have been concerned with indoor environments of residences and offices which may not necessarily be comparable to industrial or semi-industrial situations such as wood chip storage facilities. It has also become apparent that it is not practical to set standards, e.g. for total fungal CFUs/m^3. It appears to be more important to identify dangerous microorganisms in the air which are potentially pathogenic or toxigenic.

For the aerobiological assessment of residences, Miller et al. (1988) proposed the following guidelines for fungal propagules:

- CFU/m^3 of more than 50 are of concern and require further investigation if only one species is present.

- CFU/m^3 values less than 150 are acceptable if there is a mixture of species other than pathogens and certain toxigenic species.

TABLE 3

Occupational Hypersensitivity Diseases of the Lungs.

Disease	Source of Antigen	Causative Agents (Confirmed or suspected)	Reference
Farmer's Lung	Mouldy Hay	Micropolyspora faeni Thermoactinomyces vulgaris	Pepys 1969
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Sequoiosis	Mouldy sawdust	Graphium Aureobasidium pullulans	Cohen et al. 1967
Pulpmill Worker's Disease	Mouldy pulpwood	Alternaria sp.	Schlueter et al. 1972
Sawmill Worker's Lung	Mouldy wood	T. vulgaris, Aspergillus sp.	Terho et al. 1980
Wood Trimmer's Disease	Mouldy timber	Rhizopus sp. Aspergillus fumigatus	Wimander and Belin 1980
Woodchip Handler's Disease	Mouldy wood chips	Aspergillus sp., Mucor sp., M. faeni	Lundgren and Rosenhall 1979

- CFU/m^3 values up to 300 are acceptable if the species present are mainly Cladosporium or other common phylloplane fungi.

In applying these suggested guidelines to environments such as wood chip storage rooms, one has to consider the following:

Since wood chips are colonized by a wide range of microbes, the surrounding air will always contain a mixture of organisms. A survey of the literature shows that among the microorganisms growing on wood chips there are almost always fungi, actinomycetes and bacteria which are potentially pathogenic or toxigenic. Although fungi from the outside air such as Cladosporium will find their way into a wood chip storage facility, they will in most cases be qualitatively and quantitatively insignificant compared to the fungi originating from the wood chip pile.

Most reports dealing with the aerobiology of storage areas for fuel wood chips describe situations where microbial concentrations in the air are very high resulting in various acute health effects of the exposed persons. However, it can be assumed that even under favourable conditions - e.g. low microbial contamination of the chips and efficient ventilation - the microbiology of the surrounding air is very unlikely to meet the above mentioned standards for indoor environments suggested by Miller et al. At least subacute health effects can, therefore, be expected in persons who frequently and for longer periods are exposed without protection to the air in wood chip storage rooms. If the wood chips are disturbed, the concentration of microbial propagules in the air increases dramatically making adverse health

effects even more likely.

In office environments or houses, a certain concentration of microbial aerosols can be tolerated if this reflects the normal outside aerobiology or common indoor sources of microorganisms which are considered harmless. However, wood chips will almost always bear some potentially pathogenic or toxigenic microorganisms for which safe levels have not yet been established. Low concentrations of such organisms may have long-term adverse effects on healthy people or may have more severe short-term effects on persons with deficiency of the immune system.

From all this evidence, although largely indirect so far, it can be concluded that persons handling wood chips or working in wood chip storage rooms should wear protective clothing and an approved toxic dust respirator (Anonym. 1985).

Although there appears to be a fair amount of evidence that in general wood chip storage rooms are aerobiologically hazardous, it will still be necessary to conduct quantitative and qualitative analyses of the air to determine the extent of contamination. For routine checks a portable sampler which is easy to use is a practical tool. The RCS sampler has been recommended by several authors because of its ease of operation and the useful results obtained (Miller et al. 1988, Tobin et al. 1987, Samson 1985). However, since the various samplers differ widely in their efficiency and selectivity, a meaningful interpretation of the results will require the comparison of two or more sampling methods in any given situation.

The concentration of airborne microbial propagules can be extremely high in wood chip storage areas. Under such conditions some samplers such as RCS become overloaded even at the shortest practical sampling time. Samplers which can handle very high bioaerosol concentrations such as e.g. liquid impingers or filter cassettes may have to be used in these cases to obtain a realistic estimate of the microbial concentration.

The RCS sampler, as some of the other devices, becomes less efficient with decreasing size of the microbial particles to be collected. An underestimation of the smaller size fraction of the aerosol is undesirable since many of the potentially dangerous microorganisms occurring on wood chips have small spore or cell sizes. It would, therefore, be useful to compare devices which are inefficient in sampling small size particles with a sampler designed to fractionate and collect particles over a wide size range. It is generally accepted that the six-stage Anderson impactor is the best sampler for this purpose (Morey et al. 1986). However, at very high aerosol concentrations the Anderson sampler can become overloaded too and other less convenient samplers such as the All-glass-impinger, may have to be used.

In addition to a quantitative estimate of the microbial aerosol, a qualitative analysis is necessary to detect the presence of potentially harmful genera or species of microorganism. In most cases this is done by inspecting colonies on nutrient media onto which the microbial propagules have been either deposited directly during sampling (e.g. Anderson, RCS sampler) or on which the collectate from e.g. an impinger or filter cassette has been plated after appropriate dilution. For a comprehensive analysis, the use of a number of different media is

necessary depending on the types of microorganisms present. Some media are routinely used to culture airborne bacteria, actinomycetes and fungi, respectively. Some more specific media may have to be used e.g. for slow growing organisms which tend to be overgrown by faster growing species, for organisms with specific nutrient requirements or for xerophilic fungi. In addition, increased incubation temperatures have to be used to selectively grow thermophilic and thermotolerant species. Any agar-based medium can be used in conjunction with most samplers suitable for the collection of viable particles.

Depending on the history of the wood chips before or during storage, they can bear a significant amount of non-viable microbial cells, spores or fragments. These non-viable microbial particles present a health hazard as they are potentially toxigenic or allergenic. There are a number of samplers available designed to collect both viable and non-viable biological aerosols in a form that they can be inspected and analyzed microscopically.

The proper identification of microbial colonies obtained with viable cell samplers or of microbial spores, mycelia or other particles can sometimes be difficult or even impossible. Identifying to the species level requires an experienced mycologist or bacteriologist. A less in-depth analysis including some readily recognizable indicator genera can be done with the proper guidance by less experienced personnel.

Relatively simple guidelines for the identification of some common airborne fungi from the outdoor environment (Wilken-Jensen and Gravesen 1984) and from houses (Fradkin, 1985, Fradkin et al. 1987) have been published. There are also a number of key organisms which

could be used to characterize the aerobiology of wood whip storage rooms and to obtain an initial indication of a potential health hazard. However, work still needs to be done to establish a useful protocol which could be used by persons without extensive microbiological training.

2.) Storage and handling of wood chips

Microbial growth on wood chips depends on the history of the wood used for making the chips and a number of other factors. Chips made from dry roundwood, for example, are less readily colonized by microorganisms as moisture and nutrient conditions are unfavourable. On the other hand, chips made for wet wood, logging residues or whole trees provide conditions for more rapid microbial growth. These and other factors have to be kept in mind if excessive microbial contamination during chip storage is to be prevented. In general, the following recommendations can be made for the proper indoor storage and handling of wood chips:

- chips should be stored for as short a period of time as possible, not more than 1-3 months, depending on the type and condition of the raw material and the ambient temperature. They should be used on a "first in - first out" basis.

- Non-chipped wood can be stored longer without resulting in extensive microbial contamination. Therefore, it may be better, if extended storage periods are necessary, to store the wood as long as possible in the non-chipped form in a suitable location under cover.

- Chips should be kept in well ventilated storage areas and in small piles to prevent self heating and the development of the more dangerous thermophilic fungi and actinomycetes. Blowing air through the wood chip pile is an effective way to speed drying of the chips and to prevent overheating. Large chips facilitate ventilation and cooling of the pile.

- Some authors suggest that wood chips made from hardwood are more prone to microbial growth than softwood chips. However, both hardwood and softwood chips can support dangerous levels of microbial populations and equal care should be taken in the storage of both types of chips.

- Chips should be stored in a separate building or in a storage room well separated from working and living areas so that these cannot be contaminated with microbial aerosols originating from the wood chip piles.

- Storage rooms for wood chips should have an efficient separate air extraction system to avoid contaminated air reaching working or living areas An efficient ventilation system will also prevent condensation on walls and ceiling of the storage room which would favour microbial growth.

- Storage rooms or buildings should be constructed of materials which are as much as possible resistant to microbial colonization and attack such as concrete or preservative treated wood.

- Boiler and storage rooms should be kept as clean as possible to maintain a low concentration of dust in the air. When a storage room is emptied, thorough cleaning will reduce contamination of the new chip pile.

- Chemical and physical methods to prevent microbial growth have yet to be studied with regard to fuel wood chips. Treatment with chemicals may be effective but may create some environmental concerns. Airtight storage in silos or in plastic encasings appears to have a good potential to prevent microbial growth especially under long-term storage conditions.

- Persons handling wood chips or who are present in an area where wood chips are handled should wear protective clothing and respirators.

Appendix

Descriptions of Some

Common Air Microbial Samplers

The Six-Stage Anderson Sampler

Principle of Operation

Air is drawn through a circular orifice and then through a succession of six circular plates, each perforated with 400 holes, through which particles are impacted onto sterile medium in Petri dishes, placed directly beneath them (Fig. 4). Because of steadily decreasing hole diameters, the air speed reaches successively higher orifice velocities from stage 1 to stage 6. Successively smaller particles are inertially impacted onto the agar collection surfaces (stage 1: $\geq$ 7 μm, - stage 6:0.65-1.1 μm. For maximum collection efficiency a constant airstream of 1 cubic foot, or 28.3 litres per minute must be provided by a vacuum pump.

Advantages:

1. The possibility to separate particles according to their size, thereby roughly reflecting particle deposition and penetration in the upper and lower respiratory tract. Stage 1 collects spores of 10 micron in diameter and larger, on the lowest stage spores as small as 1 micron are sampled.

2. Viable spores can be determined very accurately and their numbers per cubic metre air calculated.

3. Microorganisms capable of growing on nutrient media can be detected and counted.

4. High collection efficiency, reproducibility and accuracy.

Disadvantages:

1. Requirement of an energy source, although the pump can be adjusted for battery charge.

2. Failure to detect non-growing or non-viable (dormant) spores.

3. Overgrowth of slow growing colonies by fast growing organisms, thereby making it impossible to determine the overgrown colonies.

4. In a large sample programme counting and determination are time consuming and demand qualified personnel.

Manufacturer: Anderson Samplers, Inc.
4215c Wendell Drive
Atlanta, Georgia 30336

References: Anderson (1958), Beaumont (1984)

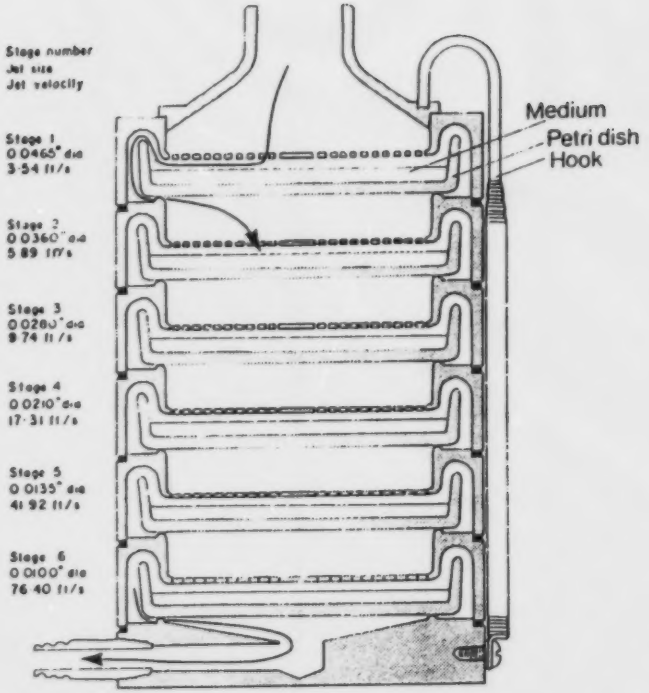

Fig. 4 The Six-Stage Anderson Sampler

The Two-Stage Anderson Sampler

Principle of Operation

The two-stage Anderson sampler operates on the same principle as the six-stage sampler. Air is drawn at a rate of 28.3 l/min through two circular plates (Fig. 5). Each plate contains two-hundred tapered orifices. The diameter of the orifices of the first stage is 1.5 mm and the diameter of the orifices of the second stage is 0.4 mm. Directly below each stage is a petri dish containing agar medium onto which particles drawn through the orifices are impacted. The sampler is designed to separate particles into two aerodynamic size ranges. Non-respirable particles are collected on stage one and respirable size particles are collected on stage two.

Advantages:

1. Easier to operate and less labour intensive than the six-stage Anderson sampler.

2. Fractionates particles into respirable and non-respirable sizes.

3. Microorganisms capable of growing on nutrient media can be detected.

Disadvantages:

1. Requirement of an energy source although a battery operated pump can be used.

2. Cannot detect non-viable or non-growing propagules.

3. Becomes easier overloaded at high aerosol concentrations than the six-stage Anderson sampler.

Manufacturer: Anderson Samplers Inc.
Atlanta, Georgia

References: Gillespie et al. (1981)

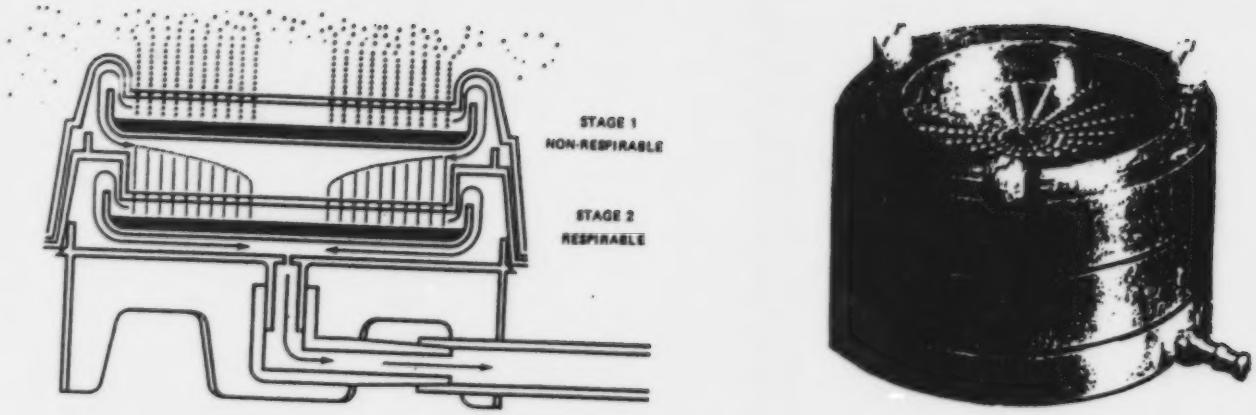

Fig. 5 The Two-Stage Anderson Sampler

The Anderson Single-Stage (N-6) Sampler

The Anderson N-6 sampler differs from the six-stage sampler in that it contains only one stage which is identical to the sixth stage of the six-stage sampler (Fig. 6). Air is drawn at a rate of 28.3 l/min through the circular plate containing 400 orifices of a diameter of 0.25 mm. Particles are impacted on the agar plate underneath the perforated plate.

Advantages:

1. Easy to operate and useful for routine air monitoring.

2. Microorganisms capable of growing on nutrient media can be collected.

Disadvantages:

1. Not fully portable since it requires an energy source, although a battery operated pump can be used.

2. Cannot detect non-viable or non-growing propagules.

3. Becomes overloaded at high aerosol concentrations.

Manufacturer: Anderson Samplers, Inc.
Atlanta, Georgia

References: Jones et al. (1985); Morey et al. (1986)

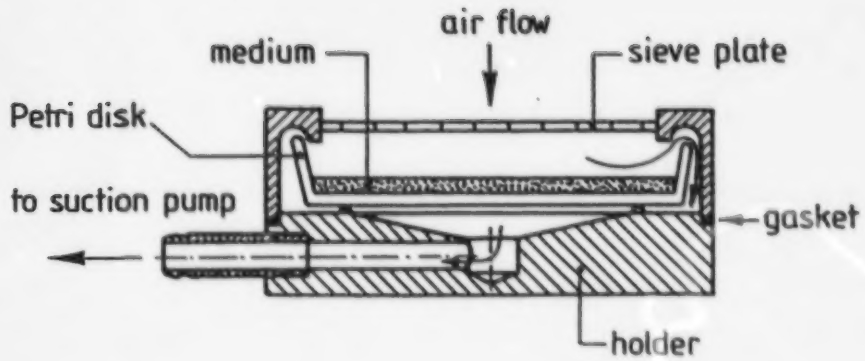

Fig. 6 The Anderson Single-Stage (N-6) Sampler

The RCS Sampler

The RCS sampler works on the impaction principle. Air is drawn into the sampler by means of an impeller (Fig. 7). Particles contained in the air are impacted by centrifugal force onto a plastic strip containing a culture medium. The sampling rate is 40 l of air per minute. After the sample has been taken the agar strips are incubated and the colonies counted.

Advantages:

1. The sampler is battery operated and portable. It weighs about 1.13 kg.

2. It is easy to operate and useful for routine monitoring.

3. Microorganisms capable of growing on the nutrient media can be collected.

Disadvantages:

1. Cannot detect non-viable or non-growing propagules.

2. Becomes overloaded at high aerosol concentrations.

3. It lacks efficiency in sampling very small particles.

Manufacturer: Biotest, Frankfurt, West Germany

References: Macher and First (1983), Canadian Mortage and Housing Corporation (1988)

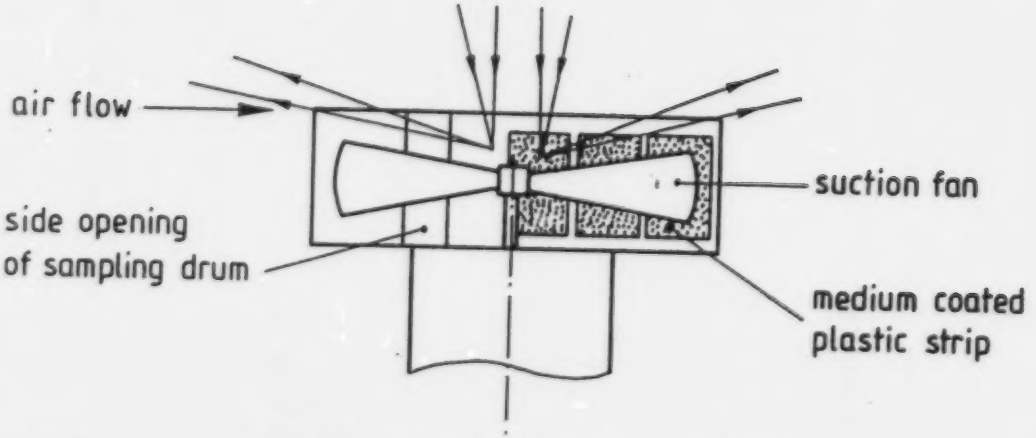

Fig. 7 The RCS Sampler and Diagram of Its Sampling Head

The SAS Sampler

Principle of Operation

Air is aspirated at a fixed speed through a detatchable cover which has been machined with multiple holes of special design (Fig. 8). Microorganisms in the air are impacted on an agar surface of a contact plate. Sampling time can be varied to adjust to the concentration of microorganisms in the air.

Advantages:

1. The sampler is battery operated and portable.
2. The operator can use any medium of his choice depending on the microorganisms present.

Disadvantages:

1. The agar surface tends to become overloaded at high concentrations of microorganisms.
2. The sampler is not very effective in collecting small cells or spores.

Manufacturer: Pool Bionalysis Haliana
Via Novara, Milano, Italy

References: Lach (1985)

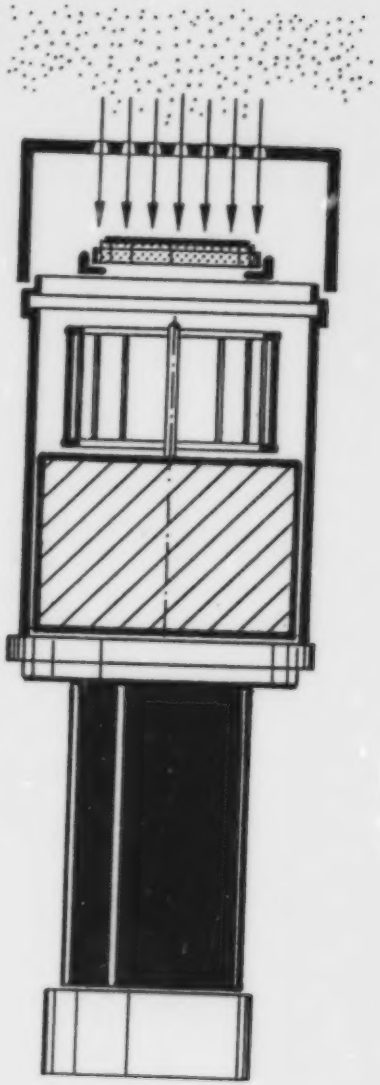

Fig. 8 The SAS Sampler and Diagram of Its Sampling Head

BIAP Slit-Sampler

Principle of Operation

The microorganisms are caught on an agar surface in a Petri dish when the air is passing a narrow slit (0.5 mm). The Petri dish rotates during the analyses and is thus exposed successively to the air (Fig. 9).

A built-in distance indicator ensures that the agar surface/slit distance is constantly 1 mm during sampling so that an optimal retention efficiency is ensured through a linear air speed of 20-25 m/sec. (33.3 L/min.).

Advantages:

1. Volumetric sampling.
2. Microorganisms capable of growing on nutrient media can be detected and counted.

Disadvantages:

1. Continuous recordings only if agar plates are removed after each sampling period.
2. Spores (organisms) must be able to germinate and grow, excludes dead organisms and obligate parasites.

Manufacturer: FOA, Umea, Sweden

References: Larsen, L.S. (1984)
Bourdillon et al. (1941)

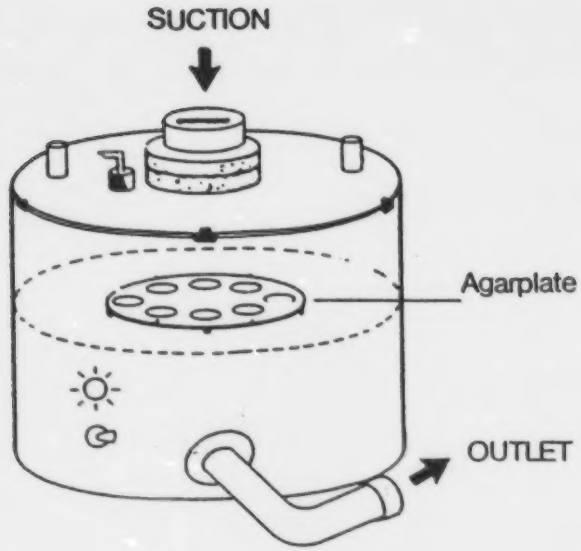

Fig. 9 Diagram of the BIAP Slit Sampler

Rotorod Sampler

Principle of Operation

The Rotorod sampler consists of a battery-operated motor equipped with a specially designed u-shaped holder at the end of the shaft (Fig. 10). Two clear acrylic rods, covered with silicone grease or vaseline as an adhesive placed in the u-shaped holders make up the collecting part of the Rotorod sampler.

At 2400 rpm the leading surfaces of the collecting rods sweep out 120 litres of air per minute.

The Rotorod sampler and its modifications collects particles in the 1 to 100 μ m size range.

Modifications:

The collecting surface in a Rotoslide sampler consists of two microscope slides inserted in upright slide holders which have replaced the rod holders in the Rotorod.

Advantages:

1. Rotorod and rotoslide samplers are cheap and easy to operate. They are especially suitable for experiments employing large numbers of simultaneous samplings.

Disadvantages:

1. Because the collecting surfaces become very easily overloaded, only short sampling periods are possible.

2. Collection efficiency is dependent on spore size and density and it is irregular over the collecting surface area. Provides total count. Does not distinguish between viable and non-viable particles.

3. When using batteries, the decreasing voltage has to be registered continuously during sampling operation and has to be taken into consideration when calculating the results.

Distributor: Metronics Associates, Inc.
Palo Alto, California

References: Rantio-Lehtinaki (1984)

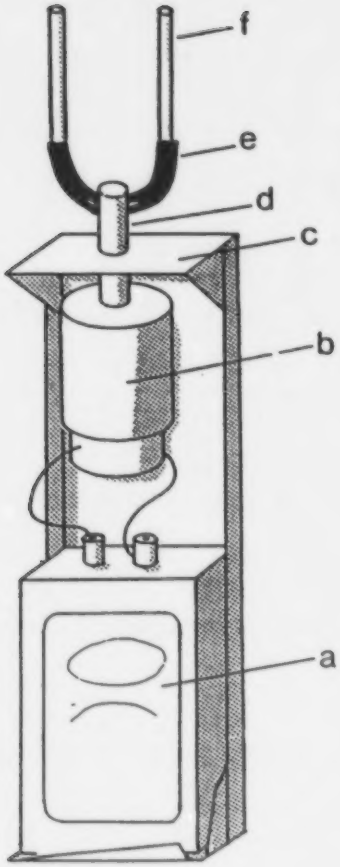

Fig. 10 Diagram of the Rotorod Sampler (a: battery, b: motor, c: housing, d: shaft, e: U-shaped rod holder, f: rod)

Burkard Spore Trap

Principle of Operation

Figure 11 demonstrates the functioning of the apparatus. A plastic strip 340 mm long and 20 mm wide is thinly smeared with Vaseline as an adhesive medium to keep the spores and the pollen grains of the air on it. The plastic strip is wound around a drum and rotated very slowly by a clockwork past a suction slid which is 14 mm long and 1.7 mm wide. The air coming in through this slit is forced onto the sticky receiving surface by means of a built-in vacuum pump in continuous operation. Air flow is 10 L/minute.

Advantages:

1. Collects non-viable as well as viable microbial propagules.

2. Time discrimination allows the recording of diurnal variations of aerosol concentrations.

3. More efficient than the rotorod sampler in collecting smaller size particles.

Disadvantages:

1. Limited portability.

2. Primarily restricted to the collection of fungal spores and pollen.

3. Not very efficient in collecting very small particles (<3 μ m).

Manufacturer: Burkard Manufacturing Co. Ltd.
Woodcock Hill, Industrial Estate
Dickmansworth, Hertfordshire, UK

References: Leuschner (1984)
Hirst (1952)

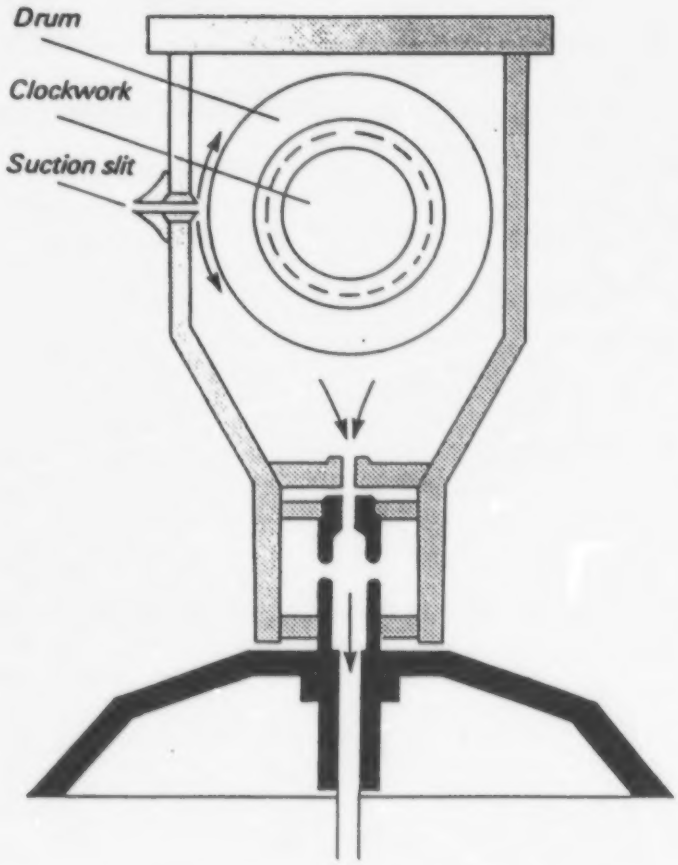

Fig. 11 Diagram of the Burkard Spore Trap

All-Glass Impinger

Principle of Operation

Air is drawn through glass tubes into the three stages of the sampler by a vacuum source (Fig. 12). Particles impact onto a submerged glass surface and elute into a buffer solution. The entry to each successive stage is smaller so that the velocity is greater and smaller particles impact. In the final stage, particles <5 μm are impinged into the solution. Suspended particles can be inspected and counted microscopically or plated on a suitable medium after appropriate dilution for CFU counts.

Advantages:

1. Suitable for collecting bacteria.

2. Efficient sampler at high aerosol concentrations.

Disadvantages:

1. Not portable. Requires heavy-duty vacuum source.

2. Not efficient in trapping hydrophobic particles. This can be overcome by adding detergents.

Manufacturer: B.G.I. Inc.
58 Guinam St., Waltham, Ma. 02154

References:
Platt-Mills et al. (1989)
May (1966)
Muilenberry (1989)

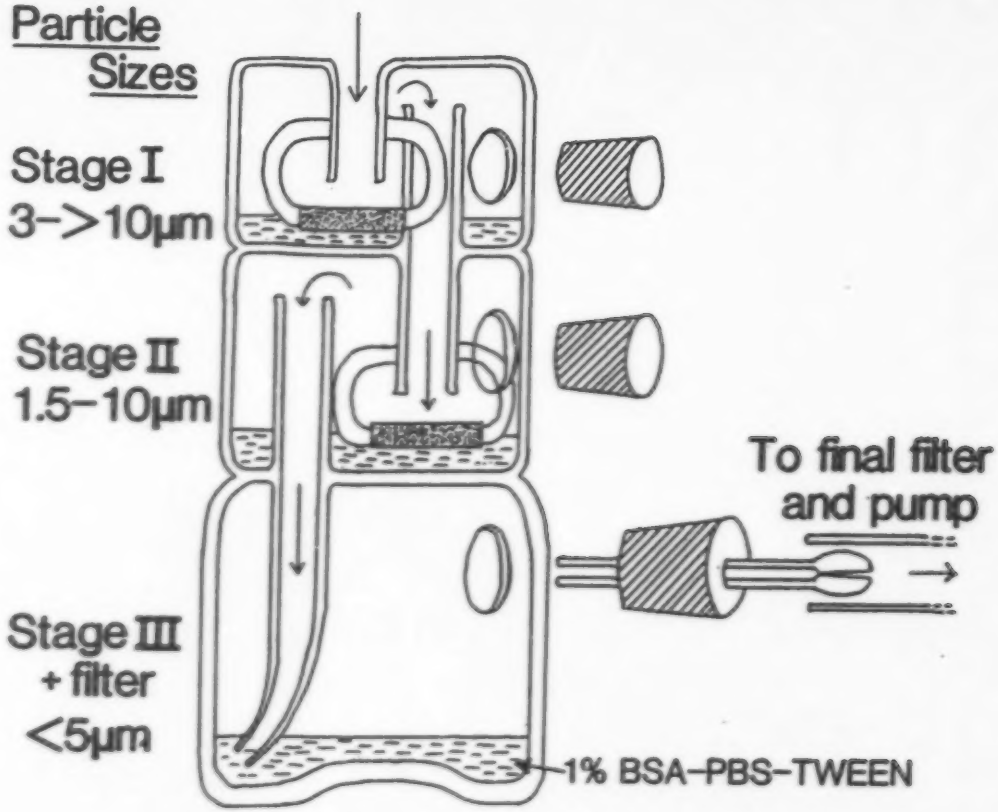

Fig. 12 Diagram of the All-Glass Liquid Impinger

Filter Cassettes

Principle of Operation

Polycarbonate membrane filters or gelatine filters with a pore size of 0.4 μm are placed and sealed in pre-sterilized plastic filter cassettes (Fig. 13). The filter holders are connected to a constant flow pump. The collected microorganisms are washed from the polycarbonate filter using a peptone/Tween 80 solution. Filters can also be inspected directly under a microscope. Gelatine filters are dissolved in peptone solution after sampling. Solutions are used for plating after appropriate dilution.

Advantages:

1. Can be used at high aerosol concentrations.

2. Useful for sampling bacteria and actinomycetes.

3. Easy to operate and portable if battery-operated pump is used.

4. Can be used to detect viable and non-viable particles.

Disadvantages:

1. Cells can be stressed during sampling by desiccation.

Manufacturers: Nucleopore Corp., Pleasanton, California

Sartorius, Gottingen, West Germany

References;

Palmgren et al. (1986)
Crook et al. (1988)
Verhoeff et al. (1988)

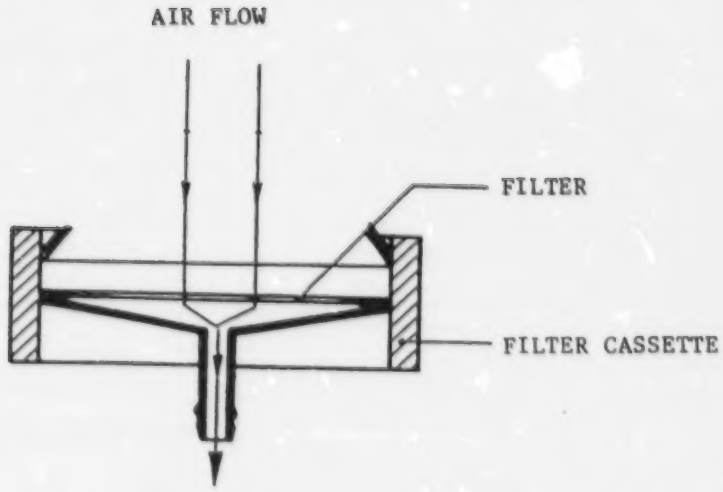

Fig. 13 Diagram of a Filter Cassette

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